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ANNUAL REPRODUCTIVE CYCLE AND SIZE AT FIRST SEXUAL MATURITY OF THE SUN AND MOON SCALLOP *AMUSIUM JAPONICUM JAPONICUM* (GMELIN, 1791) (BIVALVIA: PECTINIDAE) IN THE COASTAL WATERS OF JEJUDO, KOREA

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ABSTRACT

Gonadosomatic index (GSI), annual reproductive cycle, monthly changes in oocyte size-frequency distributions, size at first sexual maturity and sex ratio of the sun and moon scallop, *Amusium japonicum japonicum,* were investigated. Samples were collected monthly from the subtidal zone of Sogwipo, Jejudo, Korea.

Amusium japonicum japonicum is dioecious. The ovary is composed of a number of oogenic follicles, and the testis consists of a number of acini. Monthly variation in the GSI showed a pattern similar to that of the annual reproductive cycle. Ripe oocytes were about 70–90 µm in diameter. The spawning period was from November to January, and the main spawning occurred between December and January when the seawater temperature was relatively low. The annual reproductive cycle of this species can be classified into five successive stages: early active stage (April to June), late active stage (June to September), ripe stage (October to November), spawning stage (November to January), and spent / resting stage (February to April). The spawning period occurred once per year.

The percentage of first sexual maturity of female and male scallops ranging in length from 85.1 to 90.0 mm was over 50% and 100% for scallops over 90.0 mm length. In the population under study, sexually mature female and male shell lengths (50% of sexual maturity) were 86.96 and 86.59 mm respectively.

The sex ratio of individuals over 85.1 mm length was not significantly different from 1:1 ($\chi^2 = 0.18$, $p > 0.05$). No hermaphrodites were found.

Key words: *Amusium japonicum japonicum*, annual reproductive cycle, size at first sexual maturity, sex ratio.

INTRODUCTION

The sun and moon scallop, *Amusium japonicum japonicum* (Gmelin, 1791), is one of the commercially important edible scallops in East Asian countries, including Korea, China, and Japan (Yoo, 1976; Kwon et al., 1993).

The species is mainly found subtidally along the coast of Jejudo, a major southern island in Korea, inhabiting sandy bottoms to 20–40 m. As a consequence of reckless over-harvesting, the standing stock of this scallop has dramatically declined in recent years (Son et al., 1996) and the species has been denoted as a target organism and fisheries resource that should be managed using a more reasonable fishing regime (Son, 1997). For propagation and management, it is important to understand the population characteristics with regard to the reproductive cycle and size at first sexual maturity.

Several studies have examined aspects of reproduction in *Amusium* spp., including reproductive biology in *A. japonicum balloti* (Dredge, 1981), larval development (Rose et al., 1988), induced spawning and larval rearing (Beldam & del Norte, 1988; Chaitanawisuti & Menasveta, 1992), large–scale hatchery production techniques (Cropp, 1993), and aspects of ecology including growth and recruitment (Williamas & Dredge, 1981), daily growth rings (Joll, 1988), and swimming behaviour (Morton, 1980).

Regarding *Amusium japonicum japonicum*, several studies have focused on reproduction, reproductive ecology and early development (Ha, 1994), aquaculture including age and

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growth (Son et al., 1996), and spat production (Son et al., 1998). Despite the studies referred above, little information on the reproductive cycle, size at first sexual maturity, and the sex ratio of this species is available. The knowledge of the reproductive cycle, spawning period, and temporal variation in the oocyte size-frequency distributions of *A. japonicum japonicum* will provide necessary information for the determination of age and recruitment period and for the estimation of the spawning frequency during the annual spawning period. Additional information on size at first sexual maturity (rate (50%) of sexual maturity) and the sex ratio of this species would be very useful for propagation, aquaculture, and resource management. In particular, information on the size at which individuals reach first sexual maturity could be useful in determining a prohibitory measure for adequate natural resource management. In the present study the annual reproductive cycle, spawning period, size at first sexual maturity, and sex ratio of *A. japonicum japonicum* were studied.

MATERIALS AND METHODS

Sampling Methodology

Specimens of the sun and moon scallop, *A. japonicum japonicum* (Gmelin, 1791), were collected monthly by dredging in the subtidal zone of Sogwipo, Jejudo, Korea, from June 1997 to May 1999 (Fig. 1). A total of 923 scallops ranging from 20.2 to 98.1 mm length were collected during the study. Scallops were transported alive to the laboratory and gonad weight and total meat weight were measured. Unpublished data of seawater temperatures measured daily at 10:00 a.m. at South Jeju Fisheries Hatchery in Sogwipo were used for this study.

Gonadsomatic Index (GSI)

To estimate the spawning period, monthly changes in the mean gonadosomatic index (GSI) were calculated using the following formula: GSI = Gonad weight (g) x 100 / Total meat weight (g) (Chung et al., 2005).

Monthly Changes in Oocyte Size-Frequency Distributions

To investigate monthly oocyte size-frequency distributions, in each month about one thousand eggs that were cut centrally cut in the histological sections were measures, and then plotted and graphed using the frequency curve method of Pearse (1965).

Size at First Sexual Maturity

The onset of first sexual maturity was investigated histologically to determine the shell length of those specimens that developed gametes. Percentages of size at first sexual maturity were calculated as the ratio of mature individuals to the number of females and males investigated respectively.

Size at the Rate (50%) of Sexual Maturity (RM_{50})

For the investigation of age composition by size, von Bertalanffy's equation used by Son et al. (1996) is as follows: $SL_t = SL\infty\ (1- e^{-k\,(t-to)})$. Where SLt: shell length (mm) at age t; $SL\infty$: theoretical maximum shell length; k: constant expressing rate of approach to $SL\infty$; t: age t; to: theoretical age at which SL = 0 mm; TW: total weight (g) at age t.

To calculate the size at the rate (50%) of sexual maturity after fitting the rate of sexual maturity to an exponential equation, the size equivalent to the size at 50% of sexual maturity was estimated to be the sexually mature length of the population (Chung & Ryou, 2000). The exponential equation of the rate of sexual maturity is as follows: $RM = 100 / 1 + \exp^{(a-bx)}$, where, RM: rate of sexual maturity; a, b: constants; x: shell length.

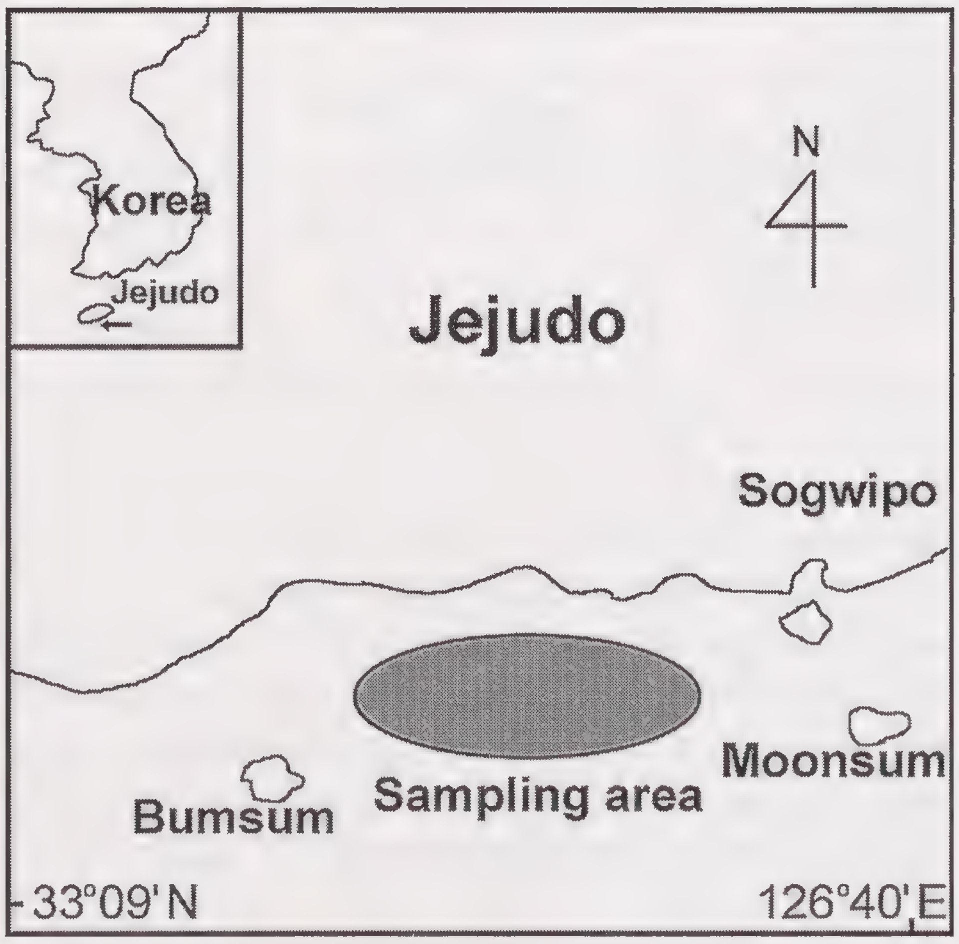

FIG. 1. Map showing the sampling area.

Sex Ratio

The sex ratio of *Amusium japonicum japonicum* was estimated from monthly samples obtained from June 1997 to May 1998; the sex of 461 sexually mature individuals > 85 mm shell length was determined by microscopic examination of histological sections. A Chi square, goodness-of-fit test was used to test the hypothesis of equal representation of females and males.

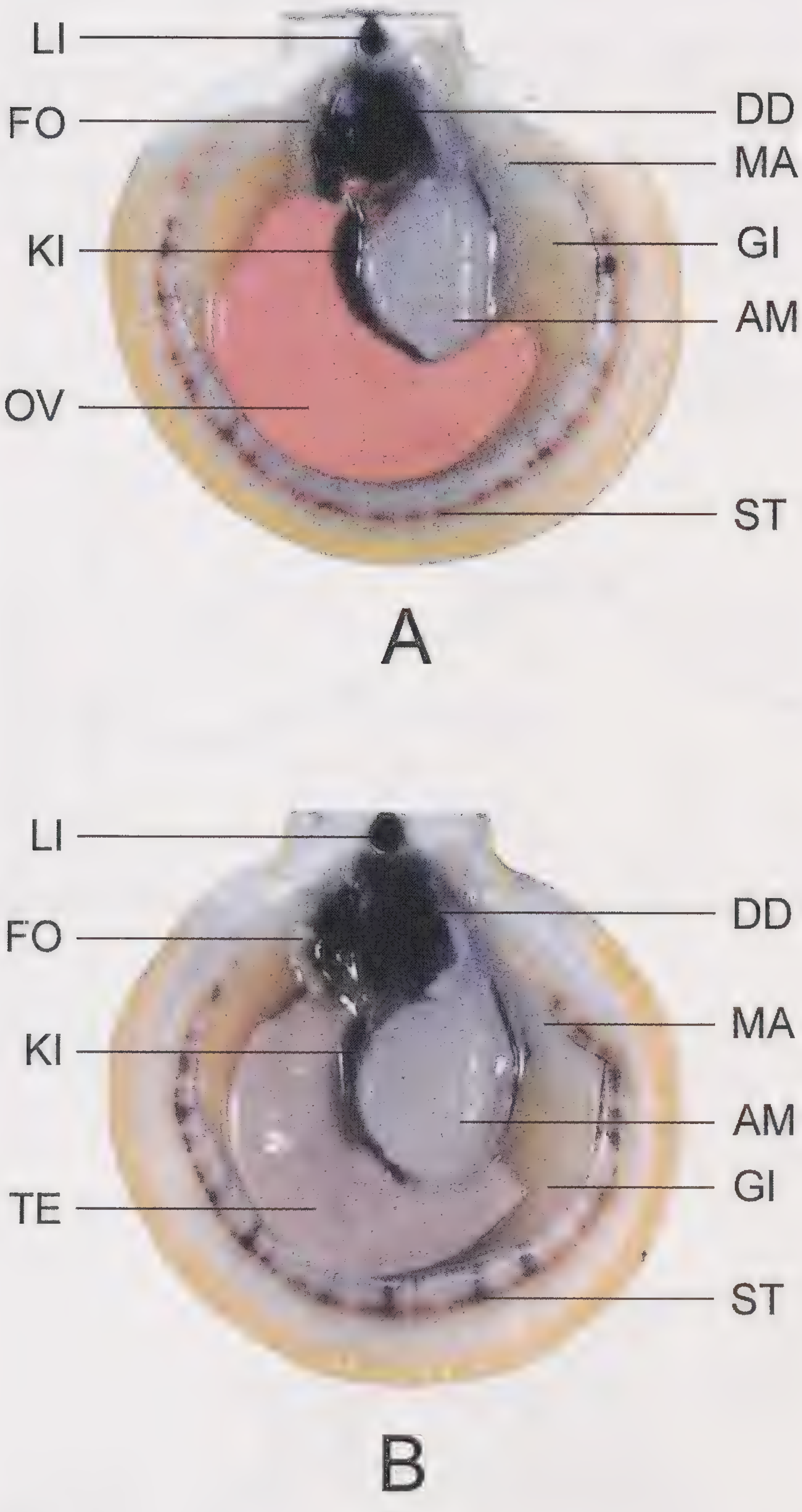

FIG. 2. Organs of mantle cavity from right side of *Amusium japonicum japonicum*, with right shell, mantle and gill removed. A: female; B: male. Abbreviations: AM, adductor muscle; DD, digestive diverticulum; FO, foot; GI, gill; KI, kidney; LI, ligament ; MA, mantle; OV, ovary; ST, sensory tentacles; TE, testis.

RESULTS

External Morphology of the Gonads

Amusium japonicum japonicum is dioecious. Gonads are conical or crescent-shaped and are well separated from the digestive diverticula lying at side of the adductor muscle. As maturity progresses, gonads encircle the adductor muscle near digestive diverticula (Fig. 2). When the gonads are mature, the external colour of the ovary is pink and that of testis is milky white or light yellow. Therefore, in sexually mature specimens the sexes can be easily determined by direct observation. After spawning, undischarged germ cells degenerate, and then it becomes difficult to distinguish the sexes by external color.

Monthly Changes in the Gonadosomatic Index (GSI) and Seawater Temperatures

Monthly GSI changes in females and males are shown in Figure 3. In 1997–1998, the values of GSI corresponding to both females and males began a gradual increase in July, reaching the maximum (females mean GSI 22.2 ± 2.5; males mean GSI 15.0 ± 1.7) in November, when seawater temperature gradually decreased. Then, the GSI rapidly decreased from December to January when relatively low water temperatures were maintained and spawning occurred continuously. Thereafter, the mean GSI value temporarily reached a minimum in May 1998 (3.4 ± 0.8) in female and June 1998 (2.5 ± 0.8) in male scallops. Monthly changes in the GSI in females and males in 1998 showed a similar pattern.

Monthly Changes in the Oocyte Size-Frequency Distributions

To determine the spawning period and the number of spawning frequencies, relative oocyte size-frequency was measured using histological sections. Monthly changes in oocyte size-frequency distributions from June 1997 to May 1998 are shown in Figure 4. Percentages of oocytes measuring more than 50 μm in diameter increased between June and July 1997. Between August and September, ripe ova measuring 70 μm in diameter increased in number.

In October 1997, the number of ripe ova rapidly began to increase and the relative frequency of ripe ova ranging from 70 to 90 µm in diameter was over 60%. In November 1997, when spawning began, the number of ripe ova measuring 70–90 µm began to decrease, however the size class of smaller egg diameter (< 20–30 µm) grew larger (> 40–60 µm). The peak spawning of ripe ova, measuring 70–90 µm in diameter, occurred continuously between December 1997 and January 1998. Thereafter, the number of ripe ova with large diameters decreased considerably in number (because of their discharge). From February to May, the small number of large undischarged oocytes, < 50–70 µm in diameter, began to degenerate and many oogonia and oocytes measuring 10–20 µm in diameter remained in the oogenic follicles.

Annual Reproductive Cycle with the Gonadal Phases

Based on morphological features and sizes of the germ cells and accompanying cells, the annual reproductive cycle of gonadal phases can be classified into five successive stages (Figs. 5, 6).

Early Active Stage: In females, oogonia and previtellogenic oocytes proliferate along the follicular wall (germinal epithelium) in the ovary. The oogonia and previtellogenic oocytes are 9–11 µm and 15–25 µm in diameter, respectively. Also some auxiliary cells, which are attached to the previtellogenic oocyte, appear near the follicular wall of the oogenic follicle (Fig. 5A).

In males, spermatogenesis occurs in acini. The spermatogonia and spermatocytes are 8–9 µm and 6–7 µm in diameter, respectively, and they appear along the acinus wall (Fig. 6A).

Female and male individuals in the early active stage appear from April to June when seawater temperatures are gradually increasing.

Late Active Stage: In females, a number of the early vitellogenic oocytes, ranging from 30

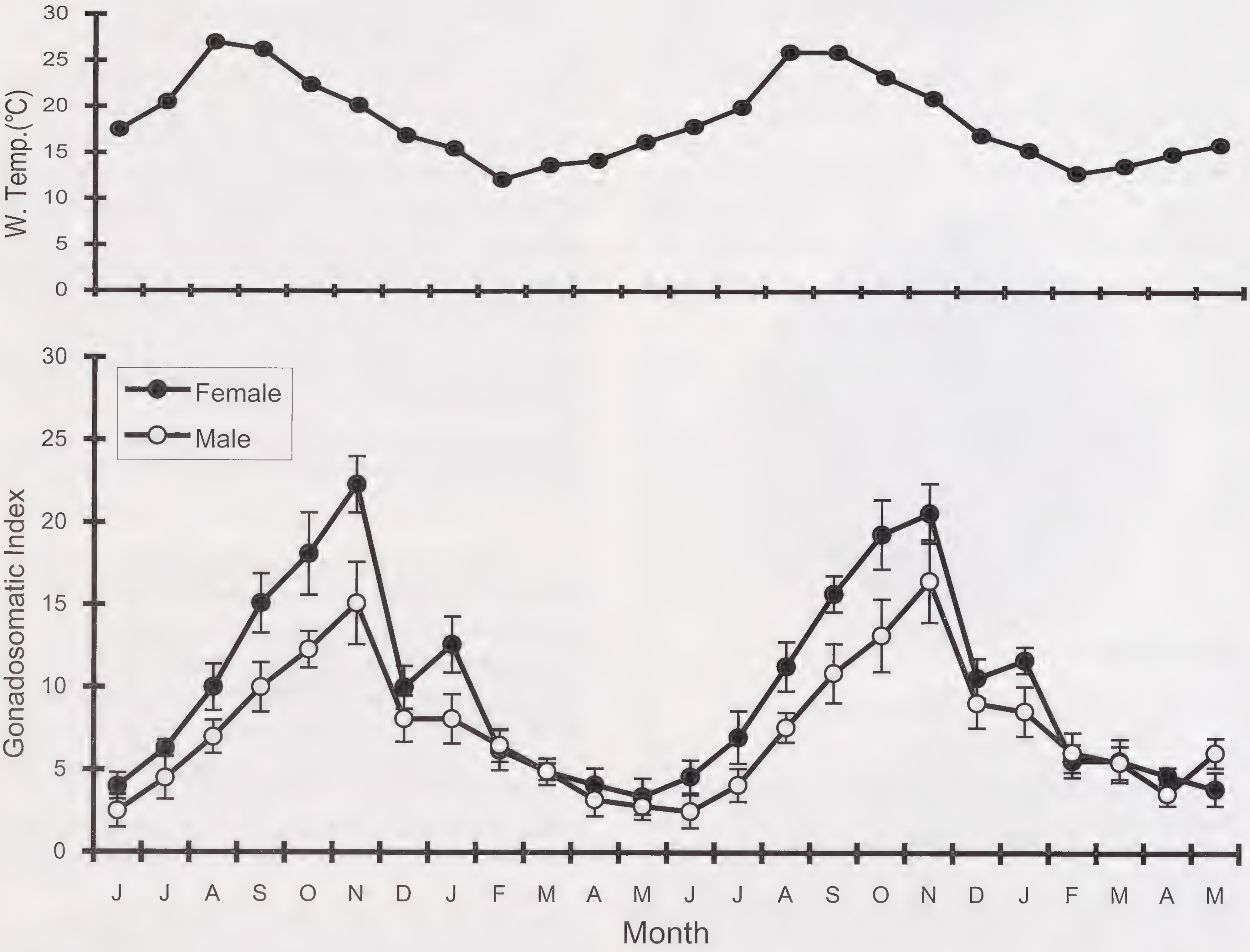

FIG. 3. Monthly variations of male and female gonadosomatic index (GSI) and mean water temperature, for two years from June 1997 to May 1999. The vertical bars indicate standard deviation (SD).

to 45 µm in diameter, appear in the oogenic follicles. Each oocyte has an egg-stalk attached to the follicular wall and the nucleus is enlarged, having a small nucleolus. At this time, some auxiliary cells, which were attached to the stalk region of an early vitellogenic oocyte, appear near the follicular wall. When late vitellogenic oocytes grow to 50–65 µm in diameter, the cytoplasm of the oocytes are filled with a number of yolk granules (Fig. 5B).

In males, a few spermatogonia and spermatocytes are present near the acinus wall. However, a small number of spermatids, measuring 3–4 µm in diameter, are also found in the lumen of the acinus. At this time a few spermatids begin to transform into differentiated spermatozoa in the centre of the lumen. As spermatogenesis progresses, the number of spermatocytes, spermatids and the few spermatozoa occupy approximately one-third to one-half of the lumina of the acini (Fig. 6B). Female and male individuals in the late active stage are found from June to September when seawater temperatures are relatively high.

Ripe Stage: In females, the ripe ova are 70–90 µm in diameter, and are round or oval in shape. They are located in the centre of the lumen. At this time, ripe ova have a large germinal vesicle; are surrounded by a thick egg envelope. The cytoplasm of the ripe ovum is filled with a number of yolk granules (Fig. 5C).

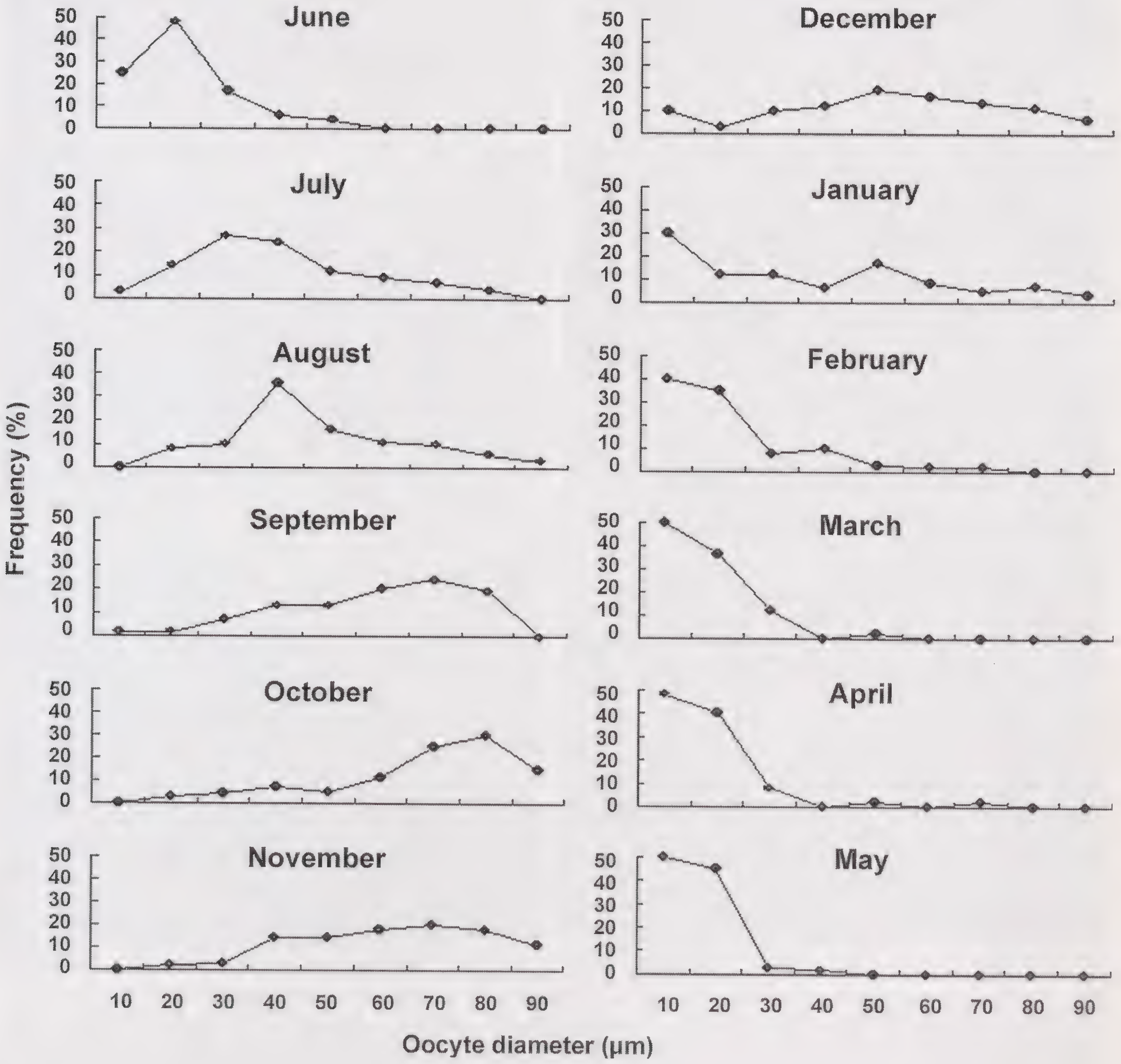

FIG. 4. Monthly changes in oocyte size-frequency distributions of *Amusium japonicum japonicum* from June 1997 to May 1998.

In males, a small number of spermatids begin to transform into differentiated spermatozoa in the centre of the lumen and numerous spermatozoa appear in the center of the lumen of the acinus (Fig. 6C). Ripe gonads are found between October and November when seawater temperatures are gradually decreasing.

Spawning Stage: In females, 50–60% of the ripe ova in the oogenic follicles have been discharged. The lumen is largely empty. Spawned ovaries are characterized by the presence of a few ripe, undischarged and previtellogenic oocytes in the lumen (Fig. 5D). In males, the lumina of the acini are empty because over 50%

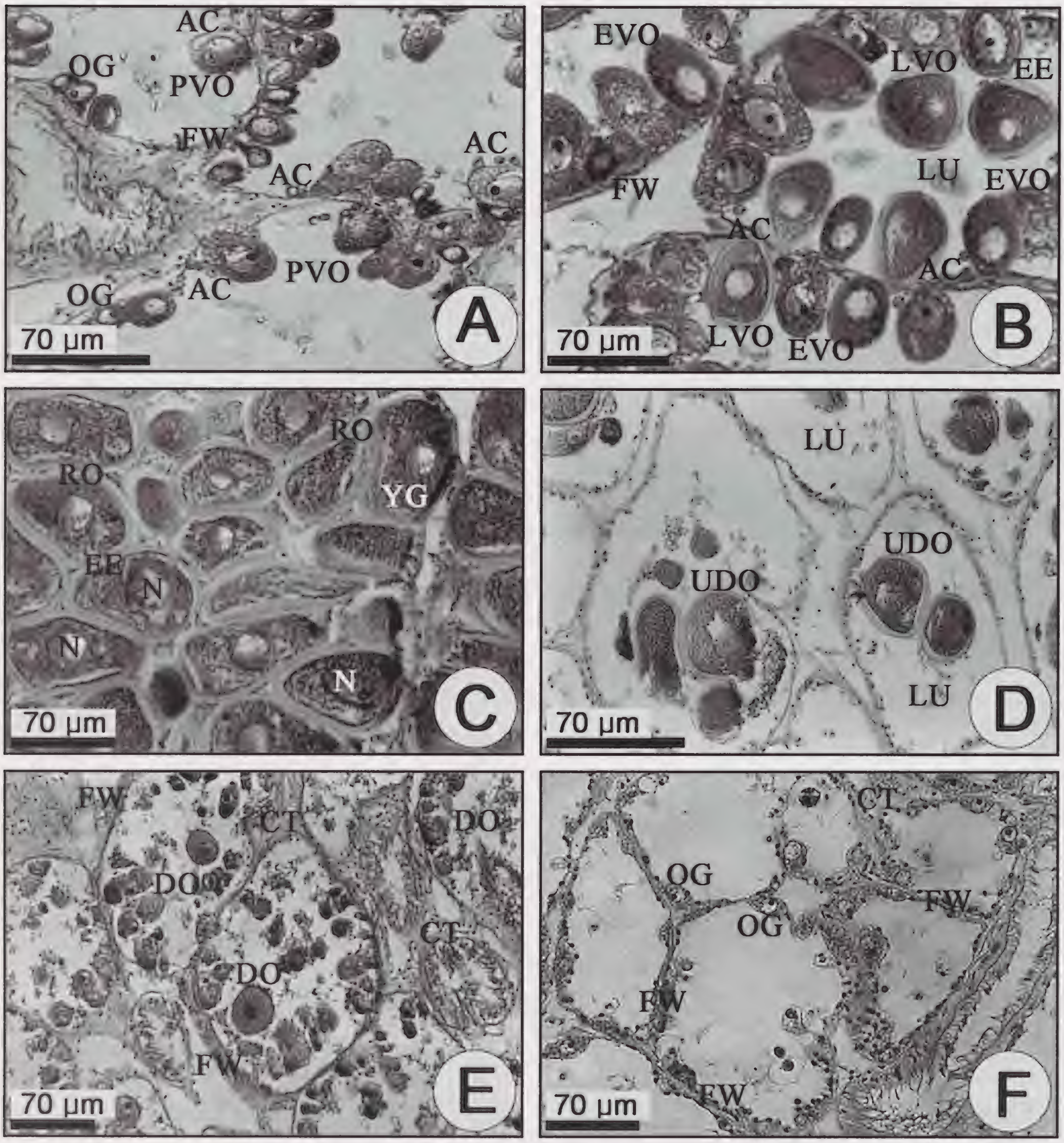

FIG. 5. Photomicrographs showing the gonadal phases in female *Amusium japonicum japonicum*. A: early active stage; B: late active stage; C: ripe stage; D: spawning stage; E, F: spent / resting stage. Abbreviations: AC, auxiliary cell; CT, connective tissue; DO, degenerating oocyte; EE, egg envelope; EVO, early vitellogenic oocyte; FW, follicular wall; LU, lumen; LVO, late vitellogenic oocyte; N, nucleus; OG, oogonium; PVO, previtellogenic oocyte; RO, ripe ovum; UDO, undischarged oocyte; YG, yolk granule.

of the spermatozoa have been discharged, but undischarged spermatozoa as well as spermatids remain in the lumen of the acinus (Fig. 6D). Individuals in the spawning stage appear from November through January, with the main spawning event occurring between December and January when seawater temperatures are relatively low.

Spent / Resting Stage: In females, after spawning, the undischarged oocytes in the lumen undergo cytolysis and the products of gamete atresia are resorbed (Fig. 5E). Thereafter, rearrangement of some new oogonia and connective tissue appeared in the oogenic follicles (Fig. 5F). In males, the few remaining spermatozoa and spermatids degenerate and

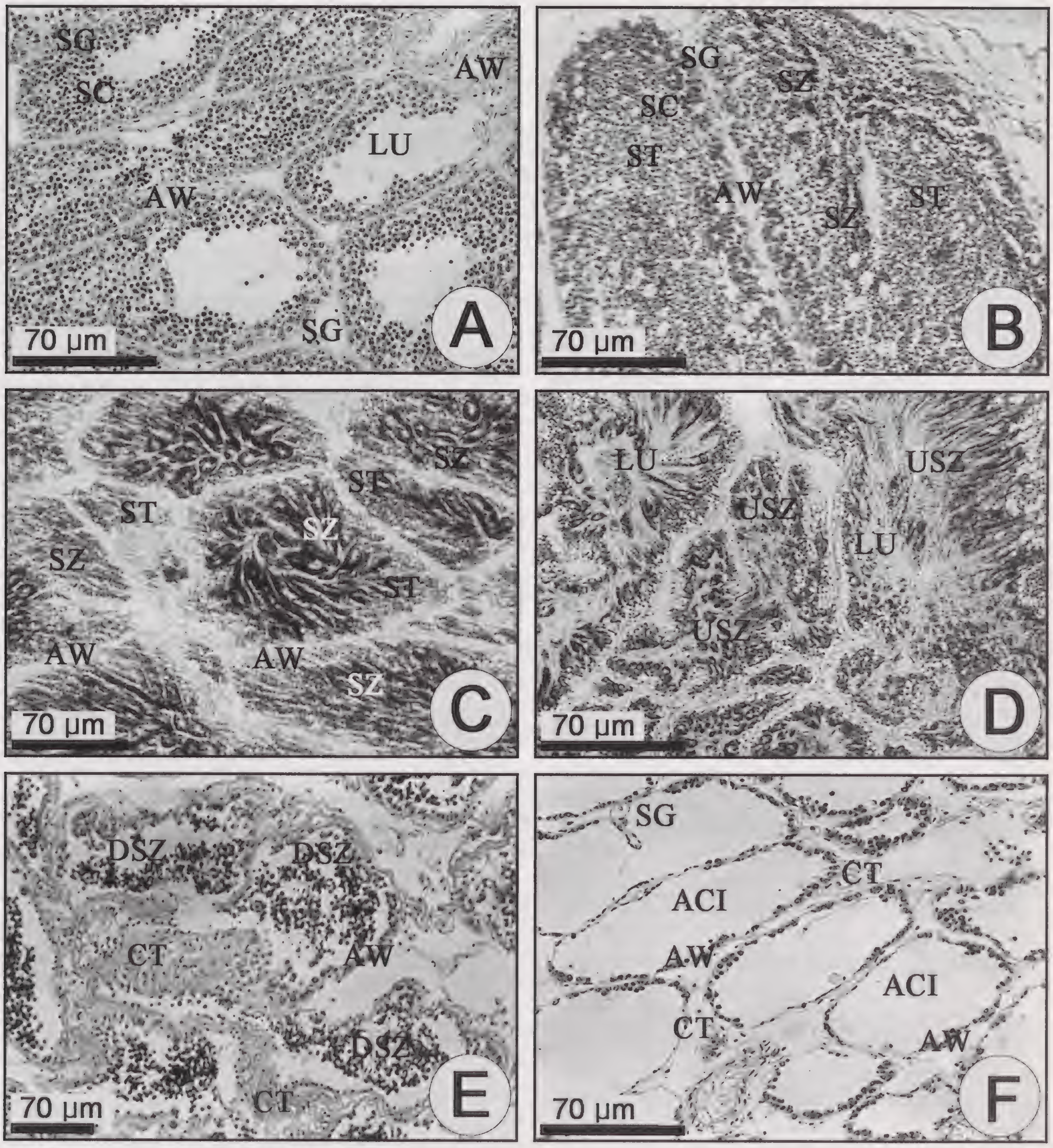

FIG. 6. Photomicrographs showing the gonadal phases in male *Amusium japonicum japonicum*. A: early active stage; B: late active stage; C: ripe stage; D: spawning stage; E, F: spent / resting stage. Abbreviations: ACI, acinus; AW, acinus wall; CT, connective tissue; DSZ, degenerating spermatozoon; LU, lumen; SC, spermatocyte; SG, spermatogonium; ST, spermatid; SZ, spermatozoon; USZ, undischarged spermatozoon.

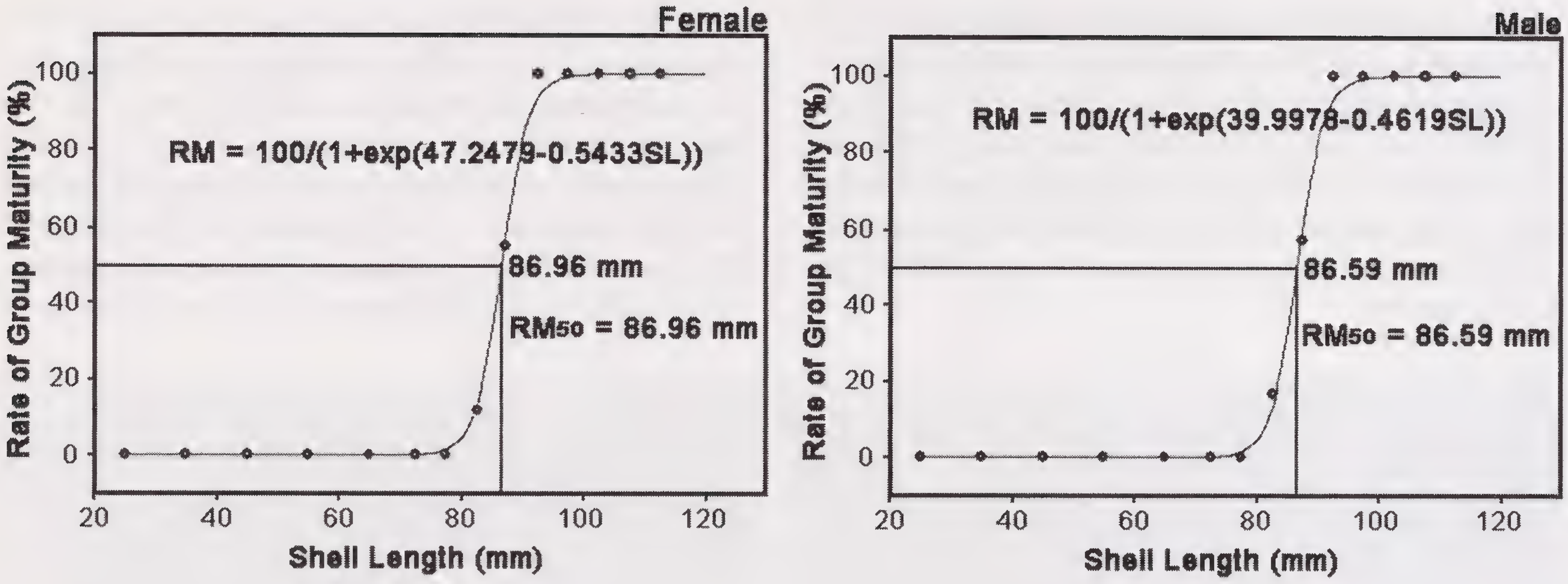

FIG. 7. Relationship between the rate of sexual maturity (%) and shell length (mm) in male and female *Amusium japonicum japonicum*.

the products of gamete atresia are resorbed (Fig. 6E). Thereafter, the rearrangement of a few newly formed spermatogonia and connective tissue occur in the acini (Fig. 6F). Female and male individuals in this stage are found from February to April when seawater temperatures are relatively low.

Size at First Sexual Maturity

A total of 171 (90 females and 81 males) individuals of *Amusium japonicum japonicum* were investigated histologically to determine the shell lengths of scallops that reach maturation and participate in reproduction from October (before spawning) to late February (after spawning). As shown in Table 1, the percentage of first sexual maturity of female and male scallops ranging in length from 85.1 to 90.0 mm were over 56%, and this was 100% for scallops over 90.1 mm length. According to the growth curves for the mean shell length fitted to von Bertalanffy's equation, individuals ranging from 85.1 to 90.0 mm in shell length were considered to be about two years old.

TABLE 1. The shell length at first sexual maturity in *Amusium japonicum japonicum*.

Shell length	Female		Male	
(mm)	Number	Mature (%)	Number	Mature (%)
20.2–30.0	4	0	4	0
30.1–40.0	7	0	5	0
40.1–50.0	7	0	4	0
50.1–60.0	6	0	6	0
60.1–70.0	7	0	7	0
70.1–75.0	8	0	6	0
75.1–80.0	8	0	8	0
80.1–85.0	9	11.1	6	16.7
85.1–90.0	9	55.5	7	57.1
90.1–95.0	7	100	6	100
95.1–100.0	7	100	4	100
100.1–105.0	4	100	5	100
105.1–110.0	4	100	7	100
110.1–114.8	3	100	6	100
Total	90		81	

TABLE 2. Monthly variations in sex ratios of the adult clam *Amusium japonicum japonicum* (> 85.1 mm in shell length).

Month	Female (ind.)	Male (ind.)	Total (ind.)	Sex ratio (F/(F+M))	Chi squared
Jun. 1997	17	22	39	0.44	0.64
Jul. 1997	16	20	36	0.44	0.44
Aug. 1997	20	18	38	0.53	0.11
Sep. 1997	22	20	42	0.52	0.10
Oct. 1997	21	19	40	0.53	0.10
Nov. 1997	22	18	40	0.55	0.40
Dec. 1997	20	17	37	0.54	0.24
Jan. 1998	24	18	42	0.57	0.86
Feb. 1998	20	17	37	0.54	0.24
Mar. 1998	21	16	37	0.57	0.68
Apr. 1998	15	21	36	0.42	1.00
May 1998	17	20	37	0.46	0.24
Total	235	226	461	0.51	0.18

Size at the Rate (50%) of Sexual Maturity (RM50)

Shell lengths of sexually mature scallops (sizes at the rate (50%) of sexual maturity, RM_{50}) that fitted to an exponential equation were 87.0 mm in females and 86.6 mm in males (Fig. 7).

Sex Ratio

Of 461 scallops examined, 235 were females and 226 were males (Table 2). There was no statistically significant difference in the numbers of females and males ($\chi^2 = 0.18$, $p > 0.05$). and similarly, monthly comparisons do not show statistical differences. In eight out of twelve months, there were slightly more females than males and opposite was tru for the remaining four months. The sex ratios of individuals over 85.0 mm in shell length were not significantly different from 1:1. No. hermaphrodites were found in histological sections.

DISCUSSION

Several authors (Pipe, 1987; Dorange & Le Pennec, 1989; Dorange et al., 1989; Gaulejac et al., 1995; Eckelbarger & Davis, 1996; Eckelbarger & Young, 1999; Chung et al., 2005) have reported that the auxiliary cells (or follicle cells), which attach to the previtellogenic and the early vitellognic oocytes in the follicle, appeared near the egg stalk region of the oocyte in the early active stage. In the present study, however, the auxiliary cells (follicle cells), which were attached to the previtellogenic and early vitellogenic oocytes, gradually detached from the late vitellogenic oocytes before the completion of the vitelline envelope during oogenesis (Pipe, 1987; Dorange et al., 1989; Gaulejac et al., 1995; Eckelbarger & Davis, 1996; Eckelbarger & Young, 1999; Chung et al., 2005).

Pipe (1987) reported that in *Mytilus edulis* once the follicle cells withdraw, the microvilli appear along the oolemma of the oocyte. In the present study, we observed a similar phenomenon. Therefore, it is assumed that the auxiliary cells, which are attached to the egg envelope of the oocyte, play some role in the formation of the vitelline envelope.

In most bivalve ovaries examined, including those of *Crassostrea virginica* (Eckelbarger & Davis, 1996), several follicle cells were associated with each oocyte during early stages of oogenesis. However, only a single “auxiliary cell” was associated with each oocyte of *Pecten maximus* (Dorange & Le Pennec, 1989; Dorange et al., 1989). In contrast, *Patinopecten yessoensis* (Chung et al., 2005) “auxiliary cells” completely encompassed oocytes (Chung et al., 2005). In the present study, a few auxiliary cells of *A. japonicum japonicum* were associated with each oocyte during early stages of oogenesis, therefore, our results showed a similar pattern to those of *P. maximus* (Dorange & Le Pennec, 1989; Dorange et al., 1989).

By using electron microscopic observation, Pipe (1987) described that the follicle cells, which are attached to the oocyte in *M. edulis*, appeared to play an integral role in oocyte development according to electron microscopic data. However, Galtsoff (1964) doubted that follicle cells played any role in oocyte nutrition in *C. virginica* due to their small size and number. Therefore, there is a need to study the functions of auxiliary cells and the relationship between the oocyte and the auxiliary cells using electron microscopy.

In marine bivalves, cyclical development and resorption of gametes can be regarded as an adaptation to environmental temperature and food availability (Morvan & Ansell, 1988). If the energy required for the production of gametes is too large, nutritive reserves might not be enough to allow all gametes to reach the critical size for spawning. In this case, the products of gamete atresia could be resorbed and the energy reallocated to still-developing oocytes or used for other metabolic purposes (Dorange & Le Pennec, 1989; Mortavkine & Varaksine, 1989).

Our histological observations showed that spawning of *Amusium japonicum* in Jejudo, Korea, occurred from November to January and the spawning peak was observed between December and January. in the present study showed that spawning of *A. japonicum japonicum* in Jejudo, Korea, occurred from November to January, and the spawning peak was observed between December and January. Judging from monthly changes in the oocyte size-frequency distributions (Fig. 4) and artificial spawning experiments in the laboratory (Son et al., 1998), the spawning period is once a year.

The spawning period of *Amusium balloti* (Bernardi, 1861) in Shark Bay, Australia, is between December and January (Heald & Caputi, 1981) and that of *A. pleuronectes* (Linnaeus, 1758) in Thailand is from November to February (Chaitanawisuti & Mennasveta, 1992). Therefore, we suggest spawning of *Amusium* spp. probably generally occurs during the period from November through February. Breeding in bivalves can occur seasonally or year-round, and as in other marine molluscs (e.g., Webber & Giese, 1969), the breeding season is affected by local environmental conditions as well as genetic differences among populations (Chung et al., 1991, 2002; Chung & Ryou, 2000). Regarding breeding habits of marine mollusks, Boolootian et al. (1962) placed mollusks into three large categories: (1) year-round breeders, (2) winter breeders, and (3) summer breeders. We found that *A. japonicum japonicum* belongs to the winter breeder class.

Son et al. (1996) found that *A. japonicum japonicum* reached a maximum shell length of 90.7 mm after two years. In the present study, percentages of first sexual maturity of female and male scallops ranging from 85.5 to 90.0 mm were 55.5% and 57.1%, respectively, and this is 100% for scallops over 90.1 mm shell length.

According to Son et al. (1996), the individuals ranging from 85.0 to 89.9 mm in shell length were considered to be about two years old. We assume that both sexes of this population begin reproduction about two years of age.

In terms of natural resource management, the present study suggests that harvesting scallops less than 85.0 mm in shell length (< 2 years old) can potentially lead to a drastic reduction in recruitment. A prohibitory measure should be taken for adequate natural resources management.

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